The Polarogram of Digested Casein by Pepsin

AUTHOR(S):

Okajima, Takashi; Sasai, Tokio

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16. The Polarogram of Digested Casein by Pepsin

Takashi OKAJIMA and Tokio SASAI

(K. Inoue Laboratory)

Although there are many reports about the method of estimation of pepsin content in the gastric juice, each has its own weak point. Therefore, we undertook the polarographic method using the protein wave. Since the appearance of protein wave is due to the SH-group of the protein molecule, we selected the casein as the substrate which contains SH-groups in its molecule. If the casein is affected by the enzymic action, its molecular structure will be altered, and the digestion products will be increased in the solution; both changes can be determined from polarographic protein wave.

In this test we used the reagents: (1) Casein solution; a definite amount of casein (Hammarsten) is dissolved in 500 ml. of 0.04 *N* hydrochloric acid by boiling. This solution is of pH 1.7 to 1.9. (2) Pepsin stock solution; 100 mg. of pure pepsin is dissolved in 1 dl. of Clark's buffer solution of pH 1.8, from which a definite dilution is made on each test. (3) Cobalt-NH_3 mixture; the cobaltic ammonium buffer solution is applied which consists of 0.001 Mole cobaltamine, 0.1 Mole ammonium chloride and 0.8 Mole ammonia. (4) 20% sulfosalicylic acid.

The procedure for digestion of casein:

To 10 ml. of casein solution was added 1 ml. of the pepsin solution variously diluted with Clark's buffer (pH 1.8), and the mixture was held in the incubator (37°C) during a definite time. Then, the sample was dipped into the boiling water for about 10 minutes.

The measurement of digestion process:

Both the denatured casein and its filtrate, which is made by addition of equivalent amount of 20 % sulfosalicylic acid, were polarographied by mixing 0.5 ml. of the sample with 5 ml. of cobalt-NH_3 mixture.

The results were as follows:

(1) The wave-height of active or inactive pepsin was much higher than that of casein. However, the wave-height of pepsin became negligible as compared with that of casein, when the proportion of their concentrations was held approximately in the order 1 : 40 (pepsin : casein).

(2) The wave-heights of casein were compared from the polarograms obtained in the condition that the concentration of each one out of three components in the cobalt-NH_3 mixture was varied, while the others being kept constant. As the results it was found that the cobaltamine had a stronger effect for the wave-height of casein than the other two components.

(3) The wave-height of denatured casein became higher than that of the native, proportionally to the concentration of pepsin up to about 100 γ/ml.; above this, however, the inverse fact was seen (Fig. 1-A). This phenomenon may be attributed to the two phases of the denaturation, namely activation and inactivation phase.

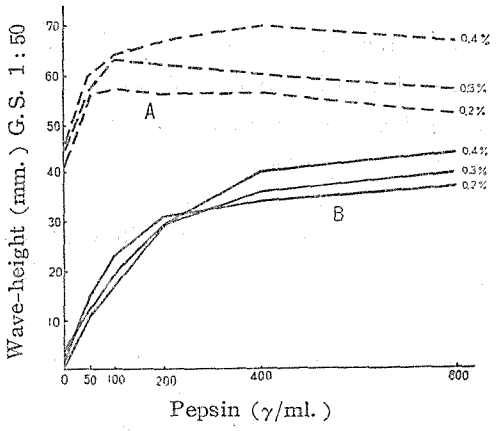

Fig. 1. Digestion of casein by pepsin.
A : the wave-height of denatured casein
B : the wave-height obtained by sulfosalicylic acid filtrate
Both tests were performed in casein solution from 0.2 to 0.4%.

The wave-height obtained from the filtrate of casein digest increased almost linearly with the pepsion concentration up to about 300 γ/ml., above this the increase became more slow (Fig. 1-B).

As to the concentration of casein, 0.4 % showed the highest protein wave for the most part, except that in the case of the filtrate 0.2 % showed the highest one for the low pepsin concentration.

(4) 0.4 % casein solution with the definite amounts of pepsin was digested from 15 to 120 minutes. Results showed that the longer the time, the higher became the wave-height, and that the inerease of wave-height in the tests at the shorter period was much more linear with the pepsin concentration than those at the longer period (Fig. 2).

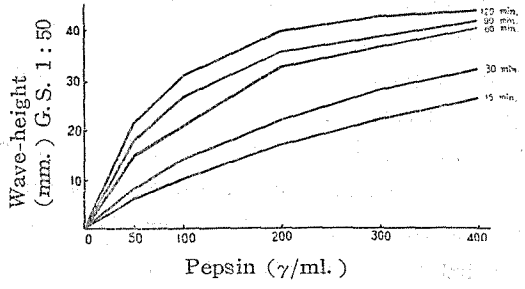

Fig. 2. Digestion of casein by pepsin in varius digesting times.

Accompanied either with the increase of pepsin concentration or with the prolongation of the digesting time, the digestion velocity of pepsin decreased gradually. This will be indicated by a fomula: $V = k[C \cdot t]^a$ (V: digestion velocity, k: constant, C: concentration of pepsin, t: digesting time, $a<1$).

In order to estimate the pepsin of unknown amount, we choosed the next conditions based on the facts above-mentioned: 0.4 % casein solution and 30 minutes of digesting time. The calibration curve of pepsin digestion, thus made, enabled us to estimate the pepsin content in the gastric juice (Fig. 3)

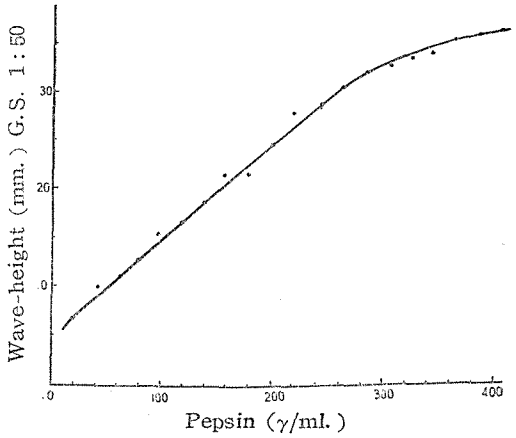

Fig. 3. The calibration curve of pepsin by protein wave.